1631

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant(s):	Dean Engelhardt et al.	
Serial No.:	08/486,069	Group Art Unit: 1631
Filed:	June 7, 1995	Ex'r: Ardin H. Marschel, Ph.D.

For: **NUCLEIC ACID SEQUENCING PROCESSES USING NON-RADIOACTIVE DETECTABLE MODIFIED OR LABELED NUCLEOTIDES OR NUCLEOTIDE ANALOGS, AND OTHER PROCESSES FOR NUCLEIC ACID DETECTION AND CHROMOSOMAL CHARACTERIZATION USING SUCH NON-RADIOACTIVE DETECTABLE MODIFIED OR LABELED NUCLEOTIDES OR NUCLEOTIDE ANALOGS (As Previously Amended)**

527 Madison Avenue (9th Floor)
New York, New York 10022
December 21, 2001

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

REQUEST FOR INTERFERENCE PURSUANT TO 37 C.F.R. §607

(1) Identification of the Patent

The patent against which applicants seek an interference is U.S. Patent No. 5,821,058, issued October 13, 1998, to Lloyd M. Smith; Leroy E. Hood; Michael W. Hunkapiller; Tim J. Hunkapiller; and Charles R. Connell ("Smith et al."). The patent purports on its face to be assigned to the California Institute of Technology, Pasadena, California.

(2) Presentation of Proposed Counts

Applicants attach hereto Appendix A which includes proposed Counts 1 and 2. Proposed Count corresponds exactly to Claim 1 of Smith et al. Proposed Count 2 corresponds exactly to Claim 41 of Smith et al. The inventions defined by the proposed counts are only two of several different inventions disclosed in the Engelhardt et al. application. However, they are appropriately used to define the subject matter of the interference because they constitute

separate patentable inventions from the other inventions disclosed in the Engelhardt et al. application.

Proposed Counts:

Count 1

A method of separating and detecting tagged polynucleotides which comprises:

providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;

resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and

detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.

Count 2

A method for detecting the sequence of a polynucleotide which comprises:

providing polynucleotide fragments generated by a polynucleotide sequencing technique, which are tagged with chromophores or fluorophores, wherein the fragments from one or more of the four sequencing reactions A, C, G or T are distinguishable from fragments of the other reaction by their spectral characteristics;

resolving the fragments by electrophoresis;

detecting the fragments as they are being resolved by means of the spectral characteristics of the chromophores or fluorophores, and thereby determining the polynucleotide sequence based on the polynucleotide fragments detected.

(3) Identifying at least one Claim in the Patent corresponding to each of the Proposed Counts

Claims 1-40 and 54-56 of the Smith et al. patent correspond to proposed Count 1. Claims 41-53 correspond to proposed Count 2. In order to assist the Examiner, applicants attach hereto Appendix B which sets forth a side-by-side comparison of (a) proposed Count 1 with independent Claims 1, 7, 14, 28, and 54 of Smith et al. and (b) proposed Count 2 with independent Claim 41 of Smith et al.

It will be appreciated that Claim 1 of Smith et al. corresponds exactly to Count 1 and Claim 41 of Smith et al. corresponds exactly to Count 2.

Although the remaining Smith et al. independent claims do not correspond exactly to the Count 1, they are nonetheless properly designated as corresponding to Count 1 because they

define the same patentable invention as Count 1. As set forth in 37 C.F.R. §1.601(n) and M.P.E.P. §2301.02:

> (n) Invention "A" is the *same patentable* invention as an invention "B" when invention "A" is the same as (35 U.S.C. 102) or is obvious (35 U.S.C. 103) in view of invention "B" assuming invention "B" is prior art with respect to invention "A." Invention "A" is a *separate patentable* invention with respect to inve ntion "B" when invention "A" is new (35 U.S.C. 102) and non-obvious (35 U.S.C. 103) in view of invention "B" assuming invention "B" is prior art with respect to invention "A."

Smith et al. Claim 7 recites the same steps as Count 1 namely, (1) providing a plurality of tagged polynucleotides; resolving the plurality of tagged polynucleotides by electrophoresis and a final detection step. The only arguable difference relates to the definition of each of the tagged polynucleotides in the providing step as being "an identical primer oligonucleotide." However, as discussed in Smith et al. columns 1 and 2, the prior art Sanger method uses such primers. Indeed, at column 4 of the Smith et al. patent, reference is made to the fact the characteristics necessary for the primers (other than the tagging aspect claimed as novel) "are satisfied by several synthetic oligonucleotide primers which are in general use for Sanger-type sequencing using M13 vectors (column 4, lines 37-39)

Smith et al. Claim 14 likewise carries out the same series of steps but specifically recites such steps as being directed to "A method of determining the sequence of a polynucleotide by analyzing polynucleotide fragments generated by a polynucleotide." Of course, in Smith et al's own words, the invention is an "Automated DNA Sequencing Technique." To the extent that the separating and detecting recited in Smith et al. Claims 1 and 7 is directed to anything other than sequencing, Smith et al. has chosen not to share such alternative applications with the public. Accordingly, because the terms separating and detecting as employed by Smith et al. are clearly directed to sequencing, the preambular differences between Claim 14 and proposed Count 1 are merely semantic ones.

Smith et al. Claim 28 likewise recites the same basic steps in a sequencing method and, therefore, also corresponds to proposed Count 1.

Finally, Smith et al. Claim 54 recites the same basic steps in the context of a sequencing method.

In view of the above, designation of Smith et al. Claims 1-40 and 54-56 as corresponding to proposed Count 1 and designation of Smith et al. Claims 41-53 as corresponding to proposed Count 2 are believed to be appropriate.

(4) Identifying at least one Claim already pending in the Application that corresponds to each of the Proposed Counts

Claims 569-709; 711-861; 863-1013; 1015-1165; 1167-1286; 1288-1297; 1700-1704; 1719-1724; 1728-1729; 1732-1748 and 1766 of Engelhardt et al. are believed to correspond to proposed Count 1. Claims 710, 862, 1014, 1166 and 1287 are believed to correspond to proposed Count 2.

To assist the Examiner in this regard, applicants attach hereto Appendix C. Appendix C is a chart providing a side-by-side comparison of the independent claims of Engelhardt et al. which correspond to proposed Count 1 and representative dependent claims of Engelhardt et al. which correspond to proposed Count 2. It will be appreciated that the claims do not correspond exactly to the proposed count, mostly by virtue of the fact that Engelhardt et al. has employed different terminology to express the same inventive concept. Nonetheless, despite the differences in language employed between the Engelhardt et al. claims and the proposed Counts 1 and 2, it is believed to be manifest that the Engelhardt et al. claims currently being designated as corresponding to the count indeed relate to the same patentable inventions as those set forth in proposed Counts 1 and 2.

Claim 569 in its literal language differs from the language of proposed Count 1 in that Claim 569 recites "A process for determining the sequence of a nucleic acid of interest" whereas the count recites a method of separating and detecting tagged polynucleotides." This represents, however, more of a semantic difference rather than a substantive difference. More specifically, the separating and detecting recited in Count 1 is precisely what is done in order to carry out the sequencing recited in Engelhardt et al. Claim 569. Indeed, Smith et al. specifically indicates at column 3, lines 8 *et seq.* that its separating and detecting related to sequencing:

> Briefly, this invention comprises a novel process for the eletrophoetic [sic, electrophoretic] analysis of DNA fragments produced in DNA, sequencing operations wherein chromophores or fluorophores are used to tag the DNA fragments produced by the sequencing chemistry and permit the detection and characterization of the fragments as they are resolved by electrophoresis through a gel.

Quite clearly, the electrophoretic analysis of DNA fragments involves separation which is followed by characterization and detection for the express purpose of sequencing. The whole focus in the objects and the examples of Smith et al. is to sequencing.

It will also be appreciated that the physical steps, including non-radioactive labeling of fragments, use of a gel, and detection of such labeled fragments are steps set forth in both Engelhardt et al. Claim 569 as well as in Count 1 (and thus in Smith et al. Claim 1).

The remaining Engelhardt et al. independent claims are also believed to correspond to Count 1 as they likewise all recite the same basic steps involving non-radioactive labeling, gel separation and detection. This is manifest from the side-by-side comparisons provided in attached Appendix C

Engelhardt et al. Claims 710, 862, 1014, 1166, 1287, 1724 and 1741 have all been designated as corresponding to Count 2. These are all dependent claims. In terms of the language employed, Claims 710, 862, 1014, 1166 and 1287 all recite that "said direct detection is carried out using one or more indicator molecules." Claim 1724 depends, alternatively, on Claims 710, 862, 1014 and 1166 and further recites that the "one or more indicator molecules" are "different indicator molecules." Claim 1741 recites that the "non-radioactively modified or labeled nucleotides or nucleotide analogs are labeled with different indicator molecules." It should be apparent that these claims set forth the same inventive concept as does Smith et al. Claim 1741, namely, the use of different indicator molecules for labeling nucleotides employed in the sequencing reaction. While Engelhardt et al.'s claims do not specifically recite the four sequencing reactions A, C, G or T, it is believed implicit that such is necessarily what Engelhardt et al. is covering in reciting different indicator molecules on the labeled nucleotides used in the sequencing reactions.

Engelhardt et al. Claims 1298-1699; 1705-1718; 1725-1727; 1730-1731 and 1749-1765 are not designated as corresponding to either Counts 1 or 2. Such claims relate to other separately patentable aspects of the invention.

(5) Applying the terms of any application claim identified as corresponding to the counts and not previously in the application to the disclosure of the application

Attached is Appendix D which sets forth where in the application the newly added claims corresponding to the proposed counts find support.

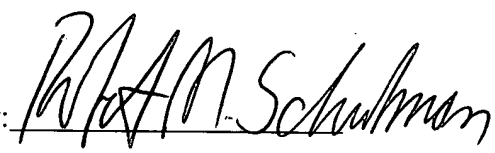
(6) Explanation as to how the Requirements of 35 U.S.C. §135(b) were met

The Smith et al. patent issued on October 13, 1998. Accordingly, compliance with 35 U.S.C. §135(b) requires that the Engelhardt et al. claims directed to the invention of the proposed Count were pending prior to October 13, 1999. In this regard, Engelhardt et al. attach hereto Appendix E which sets forth the claims that were pending in the application identified in caption as of October 13, 1999. It will be appreciated that the Engelhardt et al. claims that had been pending as of October 13, 1999, are for the same or substantially the same subject matter as the claims of the Smith et al. patent. Furthermore, although some of these claims have been amended since October 13, 1999, they likewise continued to be directed to the same or substantially the same subject matter as the claims of the Smith et al. patent.

(7) Conclusion

Applicants respectfully request that an interference be declared employing the proposed Counts 1 and 2 set forth in attached Appendix A with (1) Claims 1-40 and 54-56 of Smith et al. and Claims 569-709; 711-861; 863-1013; 1015-1165; 1167-1286; 1288-1297; 1700-1704; 1719-1724; 1728-1729; 1732-1748 and 1766 of Engelhardt et al. designated as corresponding to proposed Count 1 and Claims 41-53 of Smith et al. and Claims 710, 862, 1014, 1166, 1287, 1724 and 1741 of Engelhardt et al. designated as corresponding to proposed Count 2. Such action is respectfully requested.

Respectfully Submitted
HUNTON & WILLIAMS

By: 

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APPENDIX A

Proposed Counts

Proposed Count 1:

A method of separating and detecting tagged polynucleotides which comprises:

providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;

resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and

detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.

Proposed Count 2

A method for detecting the sequence of a polynucleotide which comprises:

providing polynucleotide fragments generated by a polynucleotide sequencing technique, which are tagged with chromophores or fluorophores, wherein the fragments from one or more of the four sequencing reactions A, C, G or T are distinguishable from fragments of the other reaction by their spectral characteristics;

resolving the fragments by electrophoresis;

detecting the fragments as they are being resolved by means of the spectral characteristics of the chromophores or fluorophores, and thereby determining the polynucleotide sequence based on the polynucleotide fragments detected.

APPENDIX B

Comparison of Smith et al. Independent Claims with the Proposed Counts

Proposed Count 1	Smith et al. Claim 1
A method of separating and detecting tagged polynucleotides which comprises:	A method of separating and detecting tagged polynucleotides which comprises:
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and	resolving to separate one of the plurality of tagged polynucleotides from other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and
detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.
Proposed Count 1	Smith et al. Claim 7
A method of separating and detecting tagged polynucleotides which comprises:	A method of detecting a tagged polynucleotide, which comprises:
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	providing a plurality of polynucleotides, wherein each of said plurality of polynucleotide comprises an identical primer oligonucleotide tagged with a chromophore or fluorophore;
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and	resolving the plurality of tagged polynucleotides using an electrophoretic procedure capable of resolving tagged polynucleotides differing in length by a single nucleotide; and
detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	detecting each of the plurality of resolved tagged polynucleotides by means of the chromophore or fluorophore.

Proposed Count 1	Smith et al. Claim 14
A method of separating and detecting tagged polynucleotides which comprises:	A method of determining the sequence of a polynucleotide by analyzing polynucleotide fragments generated by a polynucleotide sequencing technique, each of said polynucleotide fragments being tagged with a chromophore or fluorophore, comprising:
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	introducing the tagged polynucleotide fragments into an electrophoretic medium;
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and	separating the tagged polynucleotide fragments in said electrophoretic medium using an electrophoretic procedure capable of resolving said polynucleotide fragments differing in length by a single nucleotide;
detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	detecting the separated tagged polynucleotide fragments by means of the chromophore or fluorophore; and determining the polynucleotide sequence from the polynucleotide fragments detected.

Proposed Count 1	Smith et al. Claim 28
A method of separating and detecting tagged polynucleotides which comprises:	A method for determining the sequence of a polynucleotide which comprises:
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	providing polynucleotide fragments tagged with chromophores or fluorophores, wherein the chromophores or fluorophores are distinguishable from others by their spectral characteristics;
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and	resolving the polynucleotide fragments by electrophoresis; and
detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	detecting the resolved fragments by means of the chromophores or fluorophores, and thereby determining the sequence based on the polynucleotide fragments detected.

Proposed Count 2	Smith et al. Claim 41
A method for determining the sequence of a polynucleotide which comprises:	A method for determining the sequence of a polynucleotide which comprises:
providing polynucleotide fragments generated by a polynucleotide sequencing technique, which are tagged with chromophores or fluorophores, wherein the fragments from one or more of the four sequencing reactions A, C, G or T are distinguishable from fragments of the other reactions by their spectral characteristics;	providing polynucleotide fragments generated by a polynucleotide sequencing technique, which are tagged with chromophores or fluorophores, wherein the fragments from one or more of the four sequencing reactions A, C, G or T are distinguishable from fragments of the other reactions by their spectral characteristics;
resolving the fragments by electrophoresis;	resolving the fragments by electrophoresis;
detecting the fragments as they are being resolved by means of the spectral characteristics of the chromophores or fluorophores, and thereby determining the polynucleotide sequence based on the polynucleotide fragments detected. .	detecting the fragments as they are being resolved by means of the spectral characteristics of the chromophores or fluorophores, and thereby determining the polynucleotide sequence based on the polynucleotide fragments detected.
Proposed Count 1	Smith et al. Claim 54
A method of separating and detecting tagged polynucleotides which comprises:	A method of determining the sequence of a single-stranded polynucleotide comprising:
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	obtaining a set of fragments of the complement of said single-stranded polynucleotide, wherein each fragment is tagged with a chromophore or fluorophore such that fragment differing by a single nucleotide are capable of being resolved;
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and	separating the tagged fragments according to size, using an electrophoretic procedure capable of resolving tagged fragments differing by a single nucleotide, to obtain separated tagged fragments;
detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	detecting each separated tagged fragment by means of its chromophore or fluorophore; and analyzing the sizes of the separated tagged fragments, thereby determining the sequence of the single-stranded polynucleotide.

APPENDIX C

Comparison of Engelhardt *et al.* Claims with the Proposed Counts

Proposed Count 1:	Engelhardt et al. Claim 569
A method of separating and detecting tagged polynucleotides which comprises:	A process for determining the sequence of a nucleic acid of interest, comprising the steps of:
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	providing or generating detectable non-radioactively labeled nucleic acid fragments, each fragment comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactively modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, and wherein said one or more detectable non-radioactively modified or labeled nucleotides or nucleotide analogs have been modified or labeled on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the phosphate analog, the base moiety, or the base analog thereof;
	subjecting said detectable non-radioactively labeled fragments to a sequencing gel to separate or resolve said fragments; and
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and	
detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	detecting non-radioactively the presence of each of said separated or resolved fragments by means of said detectable non-radioactively modified or labeled nucleotides or nucleotide analogs, and determining the sequence of said nucleic acid of interest.

Proposed Count 1:	Engelhardt et al. Claim 721
A method of separating and detecting tagged polynucleotides which comprises:	A process for determining the sequence of a nucleic acid of interest, comprising the steps of:

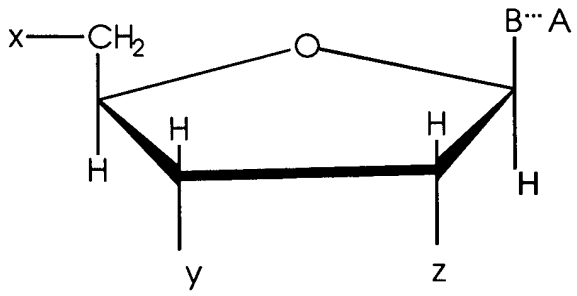
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore; resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	providing or generating detectable non-radioactively labeled nucleic acid fragments, each fragment comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactively modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, and wherein said one or more detectable non-radioactively modified or labeled nucleotides or nucleotide analogs have been modified or labeled on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the phosphate analog, the base moiety, or the base analog thereof; introducing or subjecting said detectable non-radioactively labeled fragments to a sequencing gel;separating or resolving said fragments in said sequencing gel; and detecting non-radioactively each of the separated or resolved fragments; and determining the sequence of said nucleic acid of interest.
Proposed Count 1:	Engelhardt et al. Claim 873
A method of separating and detecting tagged polynucleotides which comprises: providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	A process for determining the sequence of a nucleic acid of interest, comprising the steps of: providing or generating detectable non-radioactive labeled nucleic acid fragments, each fragment comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, and wherein said

	one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs have been modified or labeled on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the phosphate analog, the base moiety or the base analog thereof; detecting non-radioactively the detectable non-radioactive labeled nucleic acid fragments with a sequencing gel; and
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	determining the sequence of said nucleic acid of interest.
Proposed Count 1: A method of separating and detecting tagged polynucleotides which comprises: providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore; resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	Engelhardt et al. Claim 1025 A process for determining the sequence of a nucleic acid of interest, comprising the step of detecting non-radioactively with a sequencing gel one or more detectable non-radioactive labeled nucleic acid fragments comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, and wherein said one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs have been modified on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the base moiety or the base analog thereof.
Proposed Count 1: A method of separating and detecting tagged polynucleotides which comprises:	Engelhardt et al. Claim 1177 A process for determining with a sequencing gel the presence of nucleic acid fragments comprising a

providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	sequence complementary to a nucleic acid of interest or a portion thereof, said process comprising the steps of: (A) providing (i) one or more detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into a nucleic acid; or (ii) one or more oligonucleotides or polynucleotides comprising at least one said detectable non-radioactive chemically modified or labeled nucleotide or nucleotide analog; or (iii) both (i) and (ii); wherein said detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs (i) and said oligonucleotides and polynucleotides (ii) are capable of attaching to or coupling to or incorporating into or forming one or more nucleic acid fragments, and wherein said detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs have been modified or labeled non-disruptively or disruptively on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the phosphate analog, the base moiety or the base analog thereof; and; (B) incorporating said one or more detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs (i) or said one or more oligonucleotides or polynucleotides comprising at least one chemically modified or labeled nucleotides or nucleotide analogs (ii), or both (i) and (ii), into one or more nucleic acid fragments, to prepare detectable non-radioactive labeled fragments, each such fragment comprising a sequence complementary to said nucleic acid of interest or to a portion thereof and said one or more detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs, and wherein said detectable non-radioactive chemically modified or labeled nucleotides or nucleotide

modified or labeled nucleotides or nucleotide analogs are selected from the group consisting of:

(i)

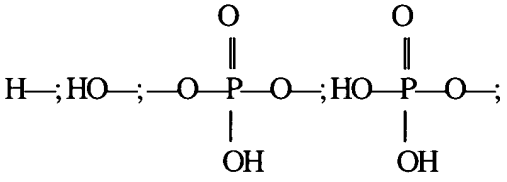


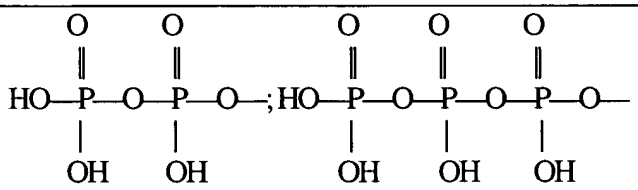
wherein B represents a purine moiety, a 7-deazapurine moiety, a pyrimidine moiety, or an analog of any of the foregoing, and B is covalently bonded to the C1-position of the sugar moiety or sugar analog, provided that whenever B is a purine, a purine analog, a 7-deazapurine moiety or a 7-deazapurine analog, the sugar moiety or sugar analog is attached at the N9 position of the purine moiety, the purine analog, the 7-deazapurine moiety or the 7-deazapurine analog thereof, and whenever B is a pyrimidine moiety or a pyrimidine analog, the sugar moiety or sugar analog is attached at the N1 position of the pyrimidine moiety or the pyrimidine analog;

wherein A comprises at least three carbon atoms and represents at least one component of a signalling moiety capable of producing directly or indirectly a detectable non-radioactive signal; and

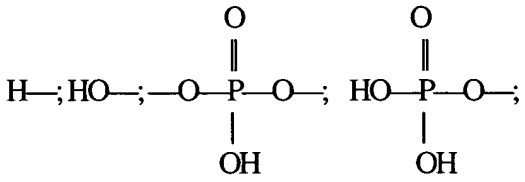
wherein B and A are covalently attached directly or through a linkage group, and

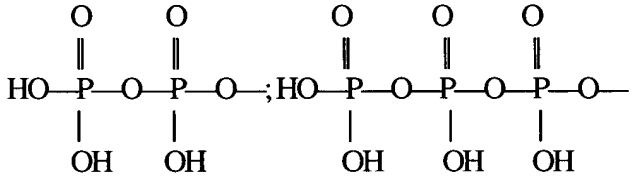
wherein x comprises a member selected from the group consisting of:





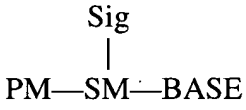
wherein y comprises a member selected from the group consisting of:





wherein z comprises a member selected from the group consisting of H– and HO–;

(ii)



wherein

PM is a phosphate moiety or phosphate analog,

SM is a sugar moiety or sugar analog,

BASE is a base moiety or base analog, and

Sig is a detectable non-radioactive moiety, and

wherein said PM is covalently attached to SM, said BASE is covalently attached to SM, and Sig is covalently attached to SM directly or through a linkage group; and

(iii)

Sig—PM—SM—BASE

	wherein PM is a phosphate moiety or phosphate analog, SM is a sugar moiety or sugar analog, BASE is a base moiety or base analog, and Sig is detectable non-radioactive moiety; and wherein PM is covalently attached to SM, BASE is covalently attached to SM, and Sig is covalently attached to PM directly or through a linkage group;
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and	(C) transferring or subjecting said detectable non-radioactive labeled fragments to a sequencing gel; (D) separating or resolving said detectable non-radioactive labeled fragments; and
detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	(E) non-radioactively detecting directly or indirectly the presence of said detectable non-radioactive labeled fragments to determine the sequence of said nucleic acid of interest.
<u>Proposed Count 1</u>:	<u>Engelhardt et. al. Claim 1700</u>
A method of separating and detecting tagged polynucleotides which comprises:	A process for determining the sequence of a nucleic acid of interest, comprising the steps of:
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	providing or generating non-radioactive labeled nucleic acid fragments, each fragment comprising a sequence complementary to said nucleic acid of interest or a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, wherein said detectable non-radioactive modified or labeled nucleotides or nucleotide analogs comprise one or more chelating compounds or chelating components capable of chelating a metal or metal ion and providing a detectable signal, and wherein said one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs have been modified or labeled on

	at least one of the sugar moiety, the sugar analog, the phosphate moiety, the phosphate analog, the base moiety, or the base analog thereof;
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and	subjecting said labeled fragments to a sequencing gel to separate or resolve said fragments; and
detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	detecting the presence of each of said separated or resolved fragments by means of the detectable signal provided by a metal or metal ion chelated by said chelating compounds or chelating components in the detectable non-radioactive modified or labeled nucleotides or nucleotide analogs, and determining the sequence of said nucleic acid of interest.
Proposed Count 1:	Engelhardt et al. Claim 1701
A method of separating and detecting tagged polynucleotides which comprises:	A process for determining the sequence of a nucleic acid of interest, comprising the steps of:
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	providing or generating detectable non-radioactive labeled nucleic acid fragments, each fragment comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, wherein said detectable non-radioactive modified or labeled nucleotides or nucleotide analogs comprise one or more chelating compounds or chelating components capable of chelating a metal or metal ion and providing a detectable signal, and wherein said one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs have been modified or labeled on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the phosphate analog, the base moiety, or the base

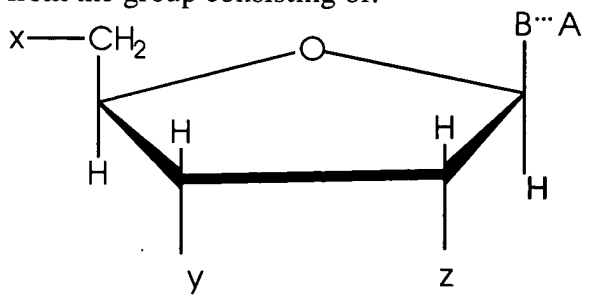
	analog thereof;
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and	introducing or subjecting said fragments to a sequencing gel; separating or resolving said fragments in said sequencing gel; and
detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	detecting each of the separated or resolved fragments by means of the detectable signal provided by a metal or metal ion chelated by said chelating compounds or chelating components in the detectable non-radioactive modified or labeled nucleotides or nucleotide analogs, and determining the sequence of said nucleic acid of interest.
Proposed Count 1:	Engelhardt et al. Claim 1702
A method of separating and detecting tagged polynucleotides which comprises:	A process for determining the sequence of a nucleic acid of interest, comprising the steps of:
providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	providing or generating detectable non-radioactive labeled nucleic acid fragments, each fragment comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, wherein said detectable non-radioactive modified or labeled nucleotides or nucleotide analogs comprise one or more chelating compounds or chelating components capable of chelating a metal or metal ion and providing a detectable signal, and wherein said one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs have been modified or labeled on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the phosphate analog, the base moiety or the base analog thereof;

resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	detecting with a sequencing gel the detectable non-radioactive labeled nucleic acid fragments by means of a metal or metal ion chelated by said chelating compounds or chelating components; and determining the sequence of said nucleic acid of interest
Proposed Count 1: A method of separating and detecting tagged polynucleotides which comprises: providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore; resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	Engelhardt et al. Claim 1703 A process for determining the sequence of a nucleic acid of interest, comprising the step of detecting with a sequencing gel one or more detectable non-radioactive labeled nucleic acid fragments comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactive modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, wherein said detectable non-radioactive modified or labeled nucleotides or nucleotide analogs comprise one or more chelating compounds or chelating components capable of chelating a metal or metal ion and providing a detectable signal, and wherein said one or more detectable non-radioactive modified nucleotides or nucleotide analogs have been modified or labeled on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the base moiety or the base analog thereof.
Proposed Count 1: A method of separating and detecting tagged polynucleotides which comprises: providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore;	Engelhardt et al. Claim 1704 A process for determining in a sequencing gel the presence of nucleic acid fragments comprising a sequence complementary to a nucleic acid sequence of interest or a portion thereof, said process comprising the steps of:

	(A) providing (i) one or more detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into a nucleic acid, or (ii) one or more oligonucleotides or polynucleotides comprising at least one of said detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs; or (iii) both (i) and (ii); wherein said detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs (i) and said oligonucleotides and polynucleotides (ii) are capable of attaching to or coupling to or incorporating into or forming one or more nucleic acid fragments, wherein said detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs comprise one or more chelating compounds or chelating components capable of chelating a metal or metal ion and providing a detectable signal, and wherein said detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs have been modified non-disruptively or disruptively on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the phosphate analog, the base moiety or the base analog thereof; and; (B) incorporating said one or more detectable non-radioactive chemically modified or labeled nucleotides or nucleotide analogs (i) or said one or more oligonucleotides or polynucleotides comprising at least one of said detectable non-radioactive chemically modified or labeled nucleotides (ii), or both (i) and (ii), into said one or more nucleic acid fragments, to prepare detectable non-radioactive labeled fragments, each such fragment comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, said detectable non-radioactive labeled fragments further comprising one or more detectable non-

radioactive chemically modified nucleotides or nucleotide analogs selected

from the group consisting of:

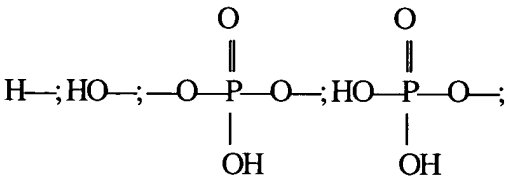


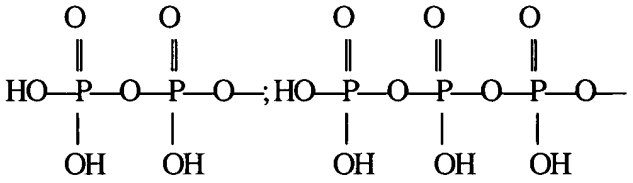
wherein B represents a purine moiety, a 7-deazapurine moiety, a pyrimidine moiety, or an analog of any of the foregoing, and B is covalently bonded to the C1'-position of the sugar moiety or sugar analog, provided that whenever B is a purine, a purine analog, a 7-deazapurine moiety or a 7-deazapurine analog, the sugar moiety or sugar analog is attached at the N9 position of the purine moiety, the purine analog, the, 7-deazapurine moiety or the 7-analog thereof, and whenever B is a pyrimidine moiety or a pyrimidine analog, the sugar moiety or sugar analog is attached at the N1 position of the pyrimidine moiety or the pyrimidine analog;

wherein A comprises at least three carbon atoms and represents at least one component of a signalling moiety comprising a chelating compound or chelating component capable of chelating a metal or metal ion and providing directly or indirectly a detectable signal; and

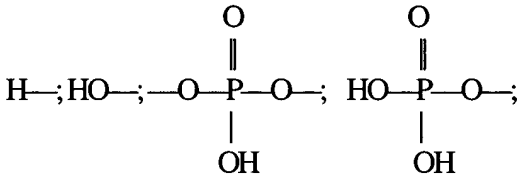
wherein B and A are covalently attached directly or through a linkage group, and

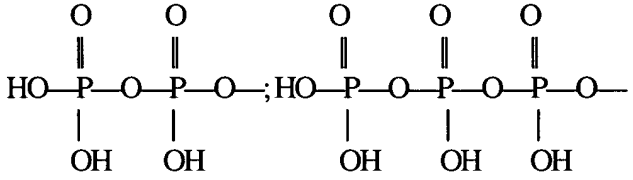
wherein x comprises a member selected from the group consisting of:





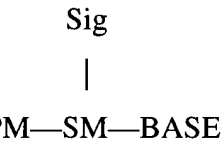
wherein y comprises a member selected from the group consisting of:





wherein z comprises a member selected from the group consisting of H- and HO-

(ii)



wherein

PM is a phosphate moiety or phosphate analog,

SM is a sugar moiety or sugar analog,

BASE is a base moiety or base analog, and

Sig is a signaling moiety comprising a chelating compound or chelating component capable of chelating a metal or metal ion and providing a detectable signal, and

wherein said PM is covalently attached to SM, said BASE is covalently attached to SM, and Sig is covalently attached to SM directly or through a linkage group; and

	(iii) Sig—PM—SM—BASE wherein PM is a phosphate moiety or phosphate analog, SM is a sugar moiety or sugar analog, BASE is a base moiety or base analog, Sig is a signaling moiety comprising a chelating compound or chelating component capable of chelating a metal or metal ion and providing a detectable signal; and wherein PM is covalently attached to SM, BASE is covalently attached to SM, and Sig is covalently attached to PM directly or through a linkage group;
resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of resolving tagged polynucleotides differing by a single nucleotide; and detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	(C) transferring or subjecting said labeled fragments to a sequencing gel; (D) separating or resolving said labeled fragments; and (E) detecting directly or indirectly the presence of said labeled fragments by means of a metal or metal ion chelated by said chelating compounds or chelating components.
Proposed Count 1:	Engelhardt et al. Claim 1766
A method of separating and detecting tagged polynucleotides which comprises: providing a plurality of polynucleotides, each tagged with a chromophore or fluorophore; resolving to separate one of the plurality of tagged polynucleotides from the other tagged polynucleotides differing in length by a single nucleotide using an electrophoretic procedure capable of	A process for determining the sequence of a nucleic acid of interest, comprising the steps of: providing or generating detectable non-radioactively labeled nucleic acid fragments, each fragment comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactively modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA; subjecting said detectable non-radioactively labeled fragments to a sequencing gel to separate or resolve said fragments; and

resolving tagged polynucleotides differing by a single nucleotide; and detecting the resolved tagged polynucleotides by means of the chromophore or fluorophore.	 detecting non-radioactively the presence of each of said separated or resolved fragments by means of said detectable non-radioactively modified or labeled nucleotides or nucleotide analogs, and determining the sequence of said nucleic acid of interest.
Proposed Count 2	Engelhardt et al. Claim 710
A method for detecting the sequence of a polynucleotide which comprises: providing polynucleotide fragments generated by a polynucleotide sequencing technique, which are tagged with chromophores or fluorophores, wherein the fragments from one or more of the four sequencing reactions A, C, G or T are distinguishable from fragments of the other reaction by their spectral characteristics; resolving the fragments by electrophoresis; detecting the fragments as they are being resolved by means of the spectral characteristics of the chromophores or fluorophores, and thereby determining the polynucleotide sequence based on the polynucleotide fragments detected.	A process for determining the sequence of a nucleic acid of interest, comprising the steps of: providing or generating detectable non-radioactively labeled nucleic acid fragments, each fragment comprising a sequence complementary to said nucleic acid of interest or to a portion thereof, wherein each of said fragments comprises one or more detectable non-radioactively modified or labeled nucleotides or nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, and wherein said one or more detectable non-radioactively modified or labeled nucleotides or nucleotide analogs have been modified or labeled on at least one of the sugar moiety, the sugar analog, the phosphate moiety, the phosphate analog, the base moiety, or the base analog thereof; subjecting said detectable non-radioactively labeled fragments to a sequencing gel to separate or resolve said fragments; and directly detecting non-radioactively the presence of each of said separated or resolved fragments by means of said detectable non-radioactively modified or labeled nucleotides or nucleotide analogs using one or more indicator molecules, and determining the sequence of said nucleic acid of interest.

APPENDIX D

Application of New Claims Corresponding to Count to Disclosure of Engelhardt *et al.*

New Engelhardt *et al.* Claims	**Engelhardt *et al.* Disclosure**
1766. A process for determining the sequence of a nucleic acid of interest, comprising the steps of:	Claim 1766 is modeled on Claim 569, and both essentially replace Claim 329, which was itself added on March 28, 1997, and later canceled. Determining the sequence of a nucleic acid is embraced by detection of labeled nucleotides, which is set forth throughout the original specification. A very non-exhaustive list of such locations includes the Title, Abstract, Background (pg. 1, 1st ¶), page 6 (penultimate ¶), page 84 (1st-3rd ¶), line 18 of page 93 to the last five lines of page 95, and Claims 1, 7, 141, 142 and 168. Of course, the term "sequence" appears throughout the specification. (See also "sequencing gel", discussed below.) Sequencing is particularly inherent in nonhybridization-based detection of labeled nucleotides. See, for example, page 103 (1st sentence of 1st full ¶), which discloses detecting single-stranded nucleic acids rather than hybrids. The inherent disclosure of "sequencing" was thoroughly addressed in the Declaration of Dr. James J. Donegan submitted with Applicants' July 6, 1998 Amendment. See Declaration beginning with page 3 (7th ¶), and page 17 (16th ¶).
providing or generating detectable non-	Providing and generating labeled nucleic acid fragments complementary to the

radioactively labeled nucleic acid fragments,

each fragment comprising a sequence

complementary to said nucleic acid of interest

or to a portion thereof,

nucleic acid of interest is a main focus of the entire specification. The specification also discloses many non-radioactive labels, and contrasts them with radioactive labels. For example, page 97, first paragraph states: "The Sig Moiety could also include a radioactive isotope component, such as radioactive cobalt, making the resulting nucleotide observable by radiation detecting means." A very non-exhaustive list of other references to radioactivity or the lack thereof appear at pages 1 (1st and 2nd ¶), 7 (1st four lines), 26 (1st ¶), 31 (1st full ¶), 37 (1st full ¶, "detection of light" as opposed to nuclear radiation), 82 (1st ¶), 84 (1st-3rd ¶), 85 (list of isotopes) and Claims 28, 37 and 38. See also Office Action of Jan. 30, 2001 (pg. 2, 3rd ¶ to pg. 4, line 1).

wherein each of said fragments comprises one

or more detectable non-radioactively modified

or labeled nucleotides or nucleotide analogs,

Nucleotide analogs are described and referred to at least 34 times in the following locations:

Page 1, 10th line from bottom
Page 7, line 9
Page 9, 2nd & 3rd lines from bottom
Page 31, line 4
Page 37, 12th line from bottom
Page 54, 2nd & 3rd full paragraphs
Page 54, last paragraph
Page 60, Example X
Page 61, Examples XI and XII
Page 62, Example XIII
Page 63, Examples XIV & XV
Page 64, Examples XVI & XVII
Page 66, Example XIX
Page 71, Example XXIII
Page 72, Examples XXIV & XXV
Page 75, Example XXXI
Page 76, Example XXXII
Page 78, Example XXXV
Page 79, Example XXXVII
Page 80, Example XXXVIII
Page 91

	Original claims 71, 72, 73, 75 & 76 See also Amendment of May 23, 2000, page 187, last paragraph to page 217, first full paragraph.
which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA;	Attachment, coupling and incorporation into DNA or RNA is disclosed throughout the entire specification, and explicitly at least at the following locations: Page 12, last paragraph Page 56, Example IV Page 57, Example V Page 58, Example VII Page 60, Example IX Page 67, Example XX Page 71, Example XXIII Page 77, Example XXXIV Page 82, first paragraph Page 99 to Page 101, first paragraph Original claims 7 & 145
subjecting said detectable non-radioactively labeled fragments to a sequencing gel	The use of a "sequencing gel" is inherently disclosed throughout the specification and explicitly on page 84, second paragraph: "This type of self-signaling molecule … is particularly important for detecting nucleic acids in gels (for example, sequencing gels)." The significance of the term "sequencing gels" was thoroughly addressed in the Donegan Declaration. See Declaration beginning with page 3 (7th ¶) and with page 17 (16th ¶). Also, the phrase "agarose-gel electrophoresis" appears in the specification on page 87 (1st full ¶).
to separate or resolve said fragments; and	Separating and resolving are disclosed throughout the specification. At least a few explicit instances of the term "resolving" or "resolution" appear on pages 31 (1st full ¶) and 33 (1st full ¶).

detecting non-radioactively the presence of each of said separated or resolved fragments by means of said detectable non-radioactively modified or labeled nucleotides or nucleotide analogs, and determining the sequence of said nucleic acid of interest.

Similarly, detecting the presence of the fragments is disclosed throughout the entire specification, essentially from page 1 to the last page, page 110, and also in the claims. A very non-exhaustive list of specific locations includes the Title, Abstract, Background (pg. 1, 1st ¶), page 6 (penultimate ¶), page 84 (1st-3rd ¶), line 18 of page 93 to the last five lines of page 95, and Claims 1, 7, 141, 142 and 168.

In conclusion, please note that the specification incorporates by reference a number of publications that provide additional support yet are not cited in this Appendix. See, for example, specification pages 50-52.

APPENDIX E

Sequencing Claims Pending Prior to October 13, 1999

329. A process for determining the sequence of a nucleic acid of interest, comprising the steps of:

providing labeled nucleic acid fragments, each fragment being complementary to a portion of or to said nucleic acid interest, wherein each of said fragments comprise one or more modified nucleotides, said modified nucleotide or nucleotides being modified on the sugar, phosphate or base moieties thereof, and comprising detectable or self-indicating labels;

subjecting said labeled fragments to a sequencing gel to separate or resolve said fragments; and

detecting the presence of each of said separated or resolved fragments by means of said detectable or self-indicating labels, thereby determining the sequence of said nucleic acid of interest.

330. The process according to claim 329, wherein said incorporating step is carried out by means of one or more primers, nucleoside triphosphates or dideoxynucleotides.

331. The process according to claims 329 or 373, wherein said modified nucleotide or nucleotides comprise a member selected from the group consisting of:

(i) a nucleotide having the formula

PM—SM—BASE—Sig

wherein

PM is a phosphate moiety,

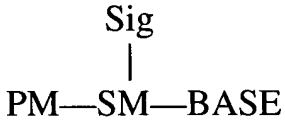
SM is a furanose moiety,

BASE is a pyrimidine, purine 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached to the furanose moiety SM at a position selected from the group consisting of the 2', the 3' and the 5' position, or any combination thereof, BASE is attached to the 1' position of SM from the N1 position when BASE is a pyrimidine or the N9 position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE at a position other than the C5 position when BASE is a pyrimidine, at a position other than the C8 position when BASE is a purine, and at a position other than C7 position when BASE is a 7-deazapurine;

(ii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety,

said PM being attached to the furanose moiety SM at a position selected from the group consisting of the 2', 3', and 5' positions, or any combination thereof, said BASE being attached to the 1' position of SM from the N1 position when BASE is a pyrimidine or the N9 position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group; and

(iii) a nucleotide having the formula

Sig—PM—SM—BASE

wherein

PM is phosphate moiety,

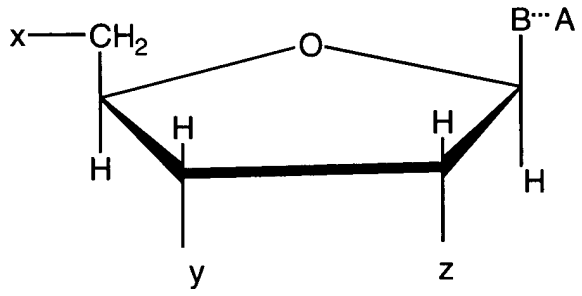
SM is furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety,

wherein PM is attached to the furanose moiety SM at a position selected from the group consisting of the 2', the 3' and the 5' position, or any combination thereof, BASE is attached to the 1' position of SM from the N1 position when BASE is a pyrimidine or the N9 position when BASE is a purine, and Sig is covalently attached to PM.

332. The process according to claims 329 or 373, wherein said modified nucleotide or nucleotides have the structure:



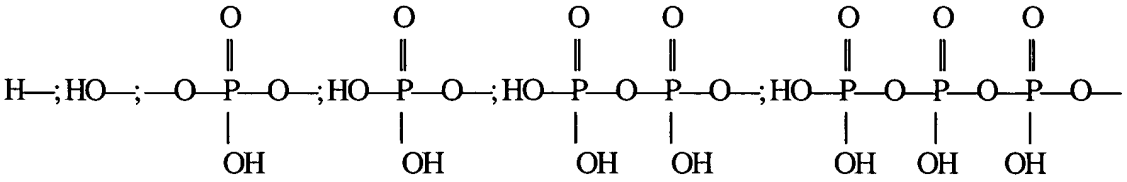
wherein B represents a purine, a 7-deazapurine or a pyrimidine moiety suitable for incorporation into a polynucleotide and covalently bonded to the C1-position of the furanose moiety, provided that when B is a purine or 7-deazapurine, the furanose moiety is attached at the N9 position of the purine or deazapurine, and when B is a pyrimidine, the furanose moiety is attached at the N1 position of the pyrimidine;

wherein A represents at least three carbon atoms and is an indicator molecule that is self-indicating;

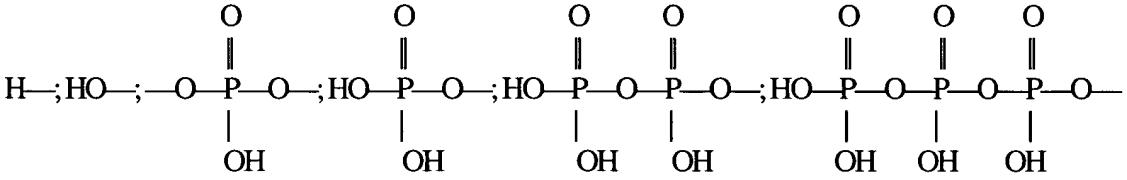
wherein B and A are covalently attached directly or through a linkage group, said linkage group not interfering substantially with detection of A;

wherein if B is a purine, A is attached to the 8-position of the purine, if B is a 7-deazapurine, A is attached to the 7-position of the deazapurine, and if B is a pyrimidne, A is attached to the 5-position of the pyrimidine; and

wherein x comprises a member selected from the group consisting of:



wherein y comprises a member selected from the group consiting of:



wherein z comprises a member selected from the group consisting of H– and HO– .

333. The process according to claims 329 or 373, wherein said self-indicating modified nucleotide comprises a member selected from the group consisting of a fluorescent component, a chemiluminescent component, and a chelating component, or a combination of any of the foregoing.

334. The process according to claims 329 or 333, wherein said labeled nucleic acid or labeled nucleic acid fragments are detectable by a means selected from the group consisting of a fluorescent measurement and a chemiluminescent measurement, or a combination thereof.

335. The process according to claim 329, wherein the labeled complementary nucleic acid is fragmented prior to separation in said sequencing gel.

336. The process according to claim 329, wherein said incorporating step, the one or more modified nucleotides are incorporated in the presence of a primer.

348. A process for determining in a sequencing gel the presence of nucleic acid fragments complementary to a nucleic acid of interest or a portion thereof, said process comprising the steps of:

(A) providing:

one or more chemically modified nucleotides capable of incorporating into a nucleic acid, alone or in conjunction with one or more other modified or unmodified nucleic acids selected from the group consisting of nucleotides, oligonucleotides and polynucleotides, said other modified or unmodified nucleic acids being capable of incorporating into or forming one or more nucleic acid fragments, each fragment being complementary to said nucleic acid of interest or to a portion thereof, said chemical modification rendering said one ore more chemically modified nucleotides either:

(I) self-indicating; or

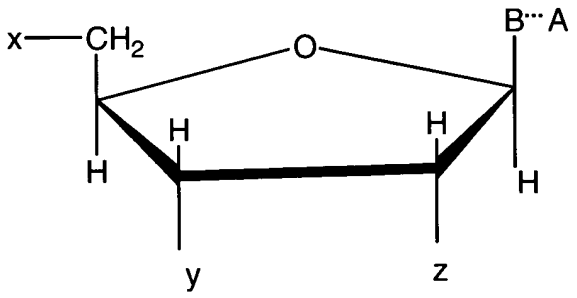
(II) comprising a label capable of providing directly or indirectly a detectable signal;

said self-indicating chemical modification or said label indicating the presence of said labeled nucleic acids or nucleic acid fragments;

said chemically modified nucleotides being modified non-disruptively or disruptively on at least one of the sugar, phosphate or base moieties thereof; and

(B) incorporating said one or more chemically modified nucleotides into said one or more fragments, thereby preparing labeled fragments, each such fragment being complementary to said nucleic acid of interest or to a portion thereof, said labeled fragments comprising one or more chemically modified nucleotides selected from the group consisting of:

(i)

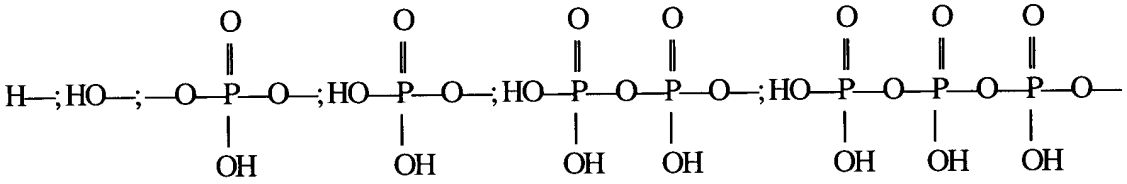


wherein B represents a purine, a 7-deazapurine or a pyrimidine moiety covalently bonded to the C1'-position of the sugar moiety, provided that whenever B is a purine or a 7-deazapurine, the sugar moiety is attached to the N9 position of the purine or 7-deazapurine, and whenever B is a pyrimidine, the sugar moiety is attached at the N1 position of the pyrimidine;

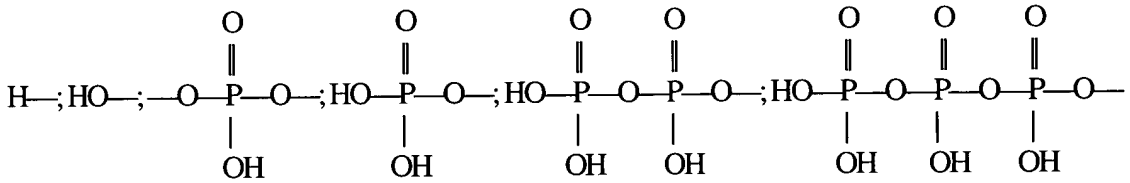
wherein A comprises at least three carbon atoms and represents at least one component of a signaling moiety capable of producing directly or indirectly a detectable signal or being self-indicating; and

wherein B and A are covalently attached directly or through a linkage group, and

wherein x comprises a member selected from the group consisting of:



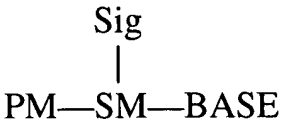
wherein y comprises a member selected from the group consisting of:



wherein z comprises a member selected from the group consisting of H– and HO–

;

(ii)



wherein

PM is a phosphate moiety,

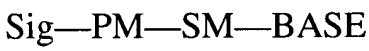
SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety or self-indicating, and

wherein said PM is attached to the furanose moiety SM at a position selected from the group consisting of the 2', 3', and 5' positions, or any combination thereof, said BASE is attached to the 1' position of SM from the N1 position when BASE is a pyrimidine or the N9 position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group; and

(iii)



wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety or is self-indicating; and

wherein PM is attached to the furanose moiety SM at a position selected from the group consisting of the 2', the 3' and the 5' position, or any combination thereof, BASE is attached to the 1' position of SM from the N1 position when BASE is a pyrimidine or the N9 position when BASE is purine, and Sig is covalently attached to PM directly or through a linkage group;

(C) transferring or subjecting said labeled fragments to a sequencing gel;

(D) separating or resolving said labeled fragments; and

(E) detecting directly or indirectly the presence of said labeled fragments.

349. The process of claim 348, wherein said incorporating step, A in the nucleotide (i) is covalently attached to B through a linkage group.

350. The process of claim 349, wherein said linkage group contains an amine.

351. The process of claim 350, wherein said amine comprises a primary amine.

352. The process of claim 348, wherein said incorporating step, Sig in the nucleotide (ii) is covalently attached to SM through a linkage group.

353. The process of claim 352, wherein said linkage group contains an amine.

354. The process of claim 353, wherein said amine comprises a primary amine.

355. The process of claim 348, wherein said incorporating step, Sig in the nucleotide (iii) is covalently attached to PM through a linkage group.

356. The process of claim 355, wherein said linkage group contains an amine.

357. The process of claim 356, wherein said amine comprises a primary amine.

358. The process of claims 349, 352 or 355, wherein said linkage group or groups do not substantially interfere with formation of the signaling moiety or detection of the detectable signal.

359. The process of claim 348, wherein said incorporating step is carried out using an enzyme.

360. The process of claim 359, wherein said enzyme comprises a polymerase.

361. The process of claim 360, wherein said polymerase comprises DNA polymerase.

362. The process of claim 348, wherein said one or more chemically modified nucleotides or said other modified or unmodified nucleic acids comprise a nucleoside di- or tri-phosphate.

363. The process of claim 348, wherein said incorporating step is template dependent or template independent.

364. The process of claim 363, wherein said incorporating step is template dependent.

365. The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide.

366. The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one terminal modified nucleotide.

367. The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide and at least one external modified nucleotide.

369. The process of claim 349, wherein said detecting step is carried out directly.

370. The process of claim 348, wherein said direct detection is carried out on one or more self-indicating nucleotides.

371. The process of claim 370, wherein said one or more self-indicating nucleotides comprise fluoresceinated nucleotides.

372. The process of claim 371, wherein said fluoresceinated nucleotides comprise fluoresceinated DNA.

373. A process for determining the sequence of a nucleic acid of interest, comprising the steps of:

providing or generating labeled nucleic acid fragments complementary to said nucleic acid of interest or to a portion thereof, each of said labeled fragments comprising one or more modified nucleotides which comprise detectable or self-indicating labels and said one or more modified nucleotides being modified on the sugar, phosphate or base moieties thereof;

introducing or subjecting said fragments to a sequencing gel;

separating or resolving said fragments in said sequencing gel; and

detecting each of the separated or resolved fragments; thereby determining the sequence of said nucleic acid of interest.

374. The process of claim 373, wherein said detecting step comprises localizing said labeled nucleic acid or said labeled nucleic acid fragments by means of said self-indicating nucleotide or nucleotides.

375. The process of claim 329, wherein said detecting step comprises localizing said labeled nucleic acid or said labeled nucleic acid fragments by means of said self-indicating nucleotide or nucleotides.

404. The process of claims 329, 348 or 373, wherein said providing step each of said nucleic acid fragments is labeled with the same or a different indicator molecule.

405. The process of claim 331, wherein said providing step each of said nucleic acid fragments is labeled with the same or a different indicator molecule.

406. The process of claim 332, wherein said providing step each of said nucleic acid fragments is labeled with the same or a different indicator molecule.

407. The process of claims 329, 348 or 373, wherein said fragments have been obtained or generated by a nucleic acid sequencing step or technique.

408. The process of claims 331, wherein said fragments have been obtained or generated by a nucleic acid sequencing step or technique.

409. The process of claims 332, wherein said fragments have been obtained or generated by a nucleic acid sequencing step or technique.

410. The process according to claim 331, wherein Sig comprises at least three carbon atoms.

411. The process according to claim 332, wherein A comprises at three carbon atoms.

412. The process according to claim 348, wherein Sig or A comprises at least three carbon atoms.

413. The process according to claim 331, wherein Sig comprises a monosaccharide, polysaccharide or an oligosaccharide.

414. The process according to claim 332, wherein A comprises a monosaccharide, polysaccharide or an oligosaccharide.

415. The process according to claim 348, wherein Sig or A comprises a monosaccharide, polysaccharide or an oligosaccharide.

416. The process according to claim 331, wherein Sig comprises a member selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme, a hormone component, a radioactive component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten, an antibody component and a chelating component.

417. The process according to claim 332, wherein A comprises a member selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an

enzyme, a hormone component, a radioactive component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten, an antibody component and a chelating component.

418. The process according to claim 348, wherein Sig or A comprises a member selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme, a hormone component, a radioactive component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten, an antibody component and a chelating component.

419. The process according to claim 416, wherein Sig comprises an electron dense component.

420. The process according to claim 417, wherein A comprises an electron dense component.

421. The process according to claim 418, wherein Sig or A comprises an electron dense component.

422. The process according to claim 419, wherein said electron dense component comprises ferritin.

423. The process according to claim 420, wherein said electron dense component comprises ferritin.

424. The process according to claim 421, wherein said electron dense component comprises ferritin.

425. The process according to claim 416, wherein Sig comprises a magnetic component.

426. The process according to claim 417, wherein A comprises a magnetic component.

427. The process according to claim 418, wherein Sig or A comprises a magnetic component.

428. The process according to claim 425, wherein said magnetic component comprises magnetic oxide or magnetic iron oxide.

429. The process according to claim 426, wherein said magnetic component comprises magnetic oxide or magnetic iron oxide.

430. The process according to claim 427, wherein said magnetic component comprises magnetic oxide or magnetic iron oxide.

431. The process according to claim 428, wherein said magnetic component comprises magnetic beads.

432. The process according to claim 429, wherein said magnetic component comprises magnetic beads.

433. The process according to claim 430, wherein said magnetic component comprises magnetic beads.

434. The process according to claim 331, wherein Sig comprises a sugar residue and the sugar residue is complexed with or attached to a sugar binding protein or a polysaccharide binding protein.

435. The process according to claim 332, wherein A comprises a sugar residue and the sugar residue is complexed with or attached to a sugar binding protein or a polysaccharide binding protein.

436. The process according to claim 348, wherein Sig or A comprises a sugar residue and the sugar residue is complexed with or attached to a sugar binding protein or a polysaccharide binding protein.

437. The process according to claim 434, wherein the binding protein comprises a lectin.

438. The process according to claim 435, wherein the binding protein comprises a lectin.

439. The process according to claim 436, wherein the binding protein comprises a lectin.

440. The process according to claim 437, wherein the lectin comprises Concanavalin A.

441. The process according to claim 438, wherein the lectin comprises Concanavalin A.

442. The process according to claim 439, wherein the lectin comprises Concanavalin A.

443. The process according to claim 437, wherein the lectin is conjugated to ferritin.

444. The process according to claim 438, wherein the lectin is conjugated to ferritin.

445. The process according to claim 439, wherein the lectin is conjugated to ferritin.

446. The process according to claim 416, wherein Sig comprises an enzyme.

447. The process according to claim 417, wherein A comprises an enzyme.

448. The process according to claim 418, wherein Sig or A comprises an enzyme.

449. The process according to claim 446, wherein the enzyme is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase, or a combination thereof.

450. The process according to claim 447, wherein the enzyme is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase, or a combination thereof.

451. The process according to claim 448, wherein the enzyme is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase, or a combination thereof.

452. The process according to claim 416, wherein Sig comprises a hormone.

453. The process according to claim 417, wherein A comprises a hormone.

454. The process according to claim 418, wherein Sig or A comprises a hormone.

455. The process according to claim 416, wherein Sig comprises a radioactive isotope.

456. The process according to claim 417, wherein A comprises a radioactive isotope.

457. The process according to claim 418, wherein Sig or A comprises a radioactive isotope.

458. The process according to claim 416, wherein Sig comprises a metal-containing component.

459. The process according to claim 417, wherein A comprises a metal-containing component.

460. The process according to claim 418, wherein Sig or A comprises a metal-containing component.

461. The process according to claim 458, wherein said metal-containing component is catalytic.

462. The process according to claim 459, wherein said metal-containing component is catalytic.

463. The process according to claim 460, wherein said metal-containing component is catalytic.

464. The process according to claim 416, wherein Sig comprises a fluorescent component.

465. The process according to claim 417, wherein A comprises a fluorescent component.

466. The process according to claim 418, wherein Sig or A comprises a fluorescent component.

467. The process according to claim 464, wherein the fluorescent component is selected from the group consisting of fluorescein, rhodamine and dansyl.

468. The process according to claim 465, wherein the fluorescent component is selected from the group consisting of fluorescein, rhodamine and dansyl.

469. The process according to claim 466, wherein the fluorescent component is selected from the group consisting of fluorescein, rhodamine and dansyl.

470. The process according to claim 416, wherein Sig comprises a chemiluminescent component.

471. The process according to claim 417, wherein A comprises a chemiluminescent component.

472. The process according to claim 418, wherein Sig or A comprises a chemiluminescent component.

473. The process according to claim 331, wherein Sig comprises an antigenic or hapten component capable of complexing with an antibody specific to the component.

474. The process according to claim 332, wherein A comprises an antigenic or hapten component capable of complexing with an antibody specific to the component.

475. The process according to claim 348, wherein Sig or A comprises an antigenic or hapten component capable of complexing with an antibody specific to the component.

476. The process according to claim 416, wherein Sig comprises an antibody component.

477. The process according to claim 417, wherein A comprises an antibody component.

478. The process according to claim 418, wherein Sig or A comprises an antibody component.

479. The process according to claim 416, wherein Sig comprises a chelating component.

480. The process according to claim 417, wherein A comprises a chelating component.

481. The process according to claim 418, wherein Sig or A comprises a chelating component.

485. The process according to claim 331, wherein Sig is detectable when it is attached to the nucleotide directly or through a linkage group.

486. The process according to claim 332, wherein A is detectable when it is attached to the nucleotide directly or through a linkage group.

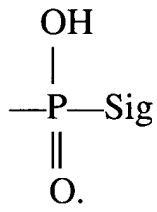
487. The process according to claim 348, wherein Sig or A is detectable when it is attached to the nucleotide directly or through a linkage group.

488. The process according to claim 485, wherein said linkage group does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

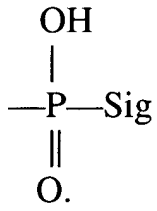
489. The process according to claim 486, wherein said linkage group does not interfere substantially with the characteristic ability of A to form a detectable signal.

490. The process according to claim 487, wherein said linkage group does not interfere substantially with the characteristic ability of Sig or A to form a detectable signal.

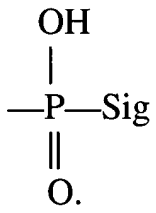
491. The process according to claim 331, wherein Sig in said nucleotide (iii) is covalently attached to PM via the chemical linkage



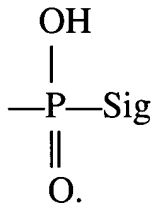
492. The process according to claims 337 or 348, wherein Sig in said nucleotide (iii) is covalently attached to PM via the chemical linkage



493. The process according to claim 331, wherein Sig in said nucleotide (iii) is covalently attached to PM via the chemical linkage



494. The process according to claims 337 or 348, wherein Sig in said nucleotide (iii) is covalently attached to PM via the chemical linkage



495. The process according to claim 331, wherein said nucleic acid fragments are terminally ligated or attached to a polypeptide.

496. The process according to claim 332, wherein said nucleic acid fragments are terminally ligated or attached to a polypeptide.

497. The process according to claim 348, wherein said nucleic acid fragments are terminally ligated or attached to a polypeptide.

498. The process according to claim 495, wherein the polypeptide comprises a polylysine.

499. The process according to claim 496, wherein the polypeptide comprises a polylysine.

500. The process according to claim 497, wherein the polypeptide comprises a polylysine.

501. The process according to claim 495, wherein the polypeptide comprises at least one member selected from the group consisting of avidin, streptavidin or anti-Sig immunoglobulin.

502. The process according to claim 496, wherein the polypeptide comprises at least one member selected from the group consisting of avidin, streptavidin or anti-Sig immunoglobulin.

503. The process according to claim 497, wherein the polypeptide comprises at least one member selected from the group consisting of avidin, streptavidin or anti-Sig immunoglobulin.

504. The process according to claim 331, wherein Sig comprises a ligand and the polypeptide comprises an antibody thereto.

505. The process according to claim 332, wherein A comprises a ligand and the polypeptide comprises an antibody thereto.

506. The process according to claim 348, wherein Sig or A comprises a ligand and the polypeptide comprises an antibody thereto.

507. The process according to claim 495, further comprising a moiety which can be detected when a complex is formed between Sig and said polypeptide, said moiety being selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme, a hormone component, a radioactive component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten, an antibody component and a chelating component.

508. The process according to claim 496, further comprising a moiety which can be detected when a complex is formed between A and said polypeptide, said moiety being selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme, a hormone component, a radioactive component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten, an antibody component and a chelating component.

509. The process according to claim 497, further comprising a moiety which can be detected when a complex is formed between Sig and said polypeptide, said moiety being selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme, a hormone component, a radioactive component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten, an antibody component and a chelating component.

520. A process for determining the sequence of a nucleic acid of interest, comprising the steps of:

providing or generating labeled nucleic acid fragments complementary to said nucleic acid of interest or to a portion thereof, each of said labeled fragments comprising one or more modified nucleotides which comprise detectable or self-indicating labels and said one or more modified nucleotides being modified on the sugar, phosphate or base moieties thereof;

detecting the labeled nucleic acid fragments within a sequencing gel; and determining the sequence of said nucleic acid of interest.

521 A process for determining the sequence of a nucleic acid of interest, comprising the step of detecting within a sequencing gel one or more labeled nucleic acid fragments complementary to said nucleic acid of interest or to a portion thereof, each of said labeled fragments comprising one or more modified nucleotides which comprise detectable or self-indicating labels, said one or more modified nucleotides being modified on the sugar, phosphate or base moieties thereof.

522. The process according to claims 520 or 521, wherein said modified nucleotide or nucleotides comprise a member selected from the group consisting of:

(i) nucleotide having the formula

PM–SM–BASE–Sig

wherein

PM is a phosphate moiety,

SM is a furanose moiety,

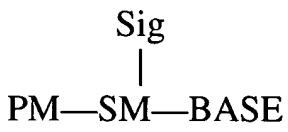
BASE is a pyrimidine, purine, 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached to the furanose moiety SM at a position selected from the group consisting of the 2', the 3' and the 5' position, or any combination there of, BASE is attached to the 1' position of SM from the N1 position when BASE is a pyrimidine or the N9 position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE at a position other than the C5 position when BASE is a

pyrimidine, at a position other than the C8 position when BASE is a purine, and at a position other than the C7 position when BASE is a 7-deazapurine;

(ii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine or 7 deazapurine, and

Sig is a detectable moiety,

said PM being attached to the furanose moiety SM at a position selected from the group consisting of the 2', 3'; and 5' positions, or any combination thereof, said BASE being attached to the 1' position of SM from the N1 position when BASE is a pyrimidine or the N9 position when BASE is a purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group; and

(iii) a nucleotide having the formula

wherein

PM is a phosphate moiety,

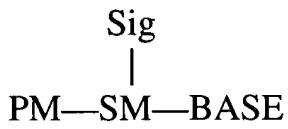
SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety,

wherein PM is attached to the furanose moiety SM at a position selected from the group consisting of the 2', the 3' and the 5' position, or any combination thereof, BASE is attached to the 1' position of SM from the N1 position when BASE is a pyrimidine or the N9 position when BASE is a purine, and Sig is covalently attached to PM.

523 The process according to claims 329, 373, 520 or 521, wherein said modified or nucleotides have the formula:



wherein

PM is a phosphate moiety,

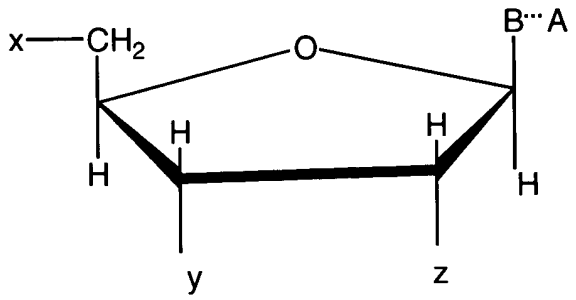
SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety,

said PM being attached being attached to the furanose moiety SM at a position selected from the group consisting of the 2', 3', and 5' positions, or any combination thereof, said BASE being attached to the 1' position of SM from the N1 position when BASE is pyrimidine or the N9 position when BASE is purine or 7-deazapurine, and Sig is covalently attached to SM directly or through a linkage group.

524. The process according to claims 520 or 521, wherein said modified nucleotide or nucleotides have the structure:



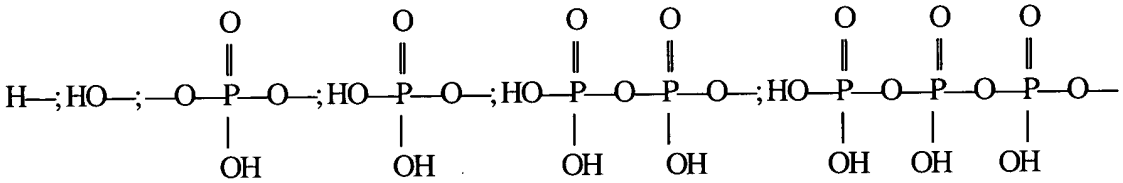
wherein B represents a purine, a 7-deazapurine or a pyrimidine moiety suitable for incorporation into a polynucleotide and covalently bonded to the C1 -position of the furanose moiety, provided that when B is a purine or 7-deazapurine, the furanose moiety is attached at the N9 position of the purine or deazapurine, and when B is a pyrimidine, the furanose moiety is attached at the N1 position of the pyrimidine;

wherein A represents at least three carbon atoms and is an indicator molecule that is self-indicating;

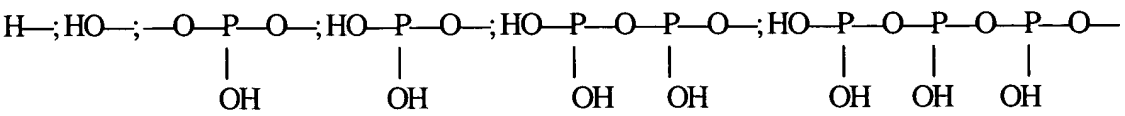
wherein B and A are covalently attached directly or through a linkage group, said linkage group not interfering substantially with detection of A;

wherein if B is a purine, A is attached to the 8-position of the purine, if B is a 7-deazapurine, A is attached to the 7-position of the deazapurine, and if B is a pyrimidine, A is attached to the 5-position of the pyrimidine; and

wherein x comprises a member selected from the group consisting of:



wherein in y comprises a member selected from the group consisting of:



wherein z comprises a member selected from the group consisting of H— and HO—.

525. The process according to claim 520, wherein the detecting step comprises localizing said labeled nucleic acid or said labeled nucleic acid fragments by means of self-indicating nucleotide or nucleotides.

526. The process according to claims 520 or 521, wherein said nucleic acid of interest comprises or is derived from *Neisseria gonorrhoeae.*

527. The process according to claim 322, wherein y and z comprise H.

528. The process according to claim 348, wherein y and z comprise H.

529. The process according to claim 524, wherein y and z comprise H.

530. The process according to claims 329, 373, 520 or 521, wherein modified nucleotide or nucleotides have the formula

Sig—PM—SM—BASE

wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety,

wherein PM is attached to the furanose moiety SM at a position selected from the group consisting of the 2', the 3' and the 5' position, or any combination thereof, BASE is attached to the 1' position of SM from the N1 position when BASE is a pyrimidine or the N9 position when BASE is a purine, and Sig is covalently attached to PM.

537. The process according to claim 331, wherein said Sig detectable moiety comprises an aliphatic chemical moiety comprising at least three carbon atoms and at least one double bond.

538. The process according to claim 331, wherein said Sig detectable moiety comprises an aliphatic chemical moiety comprising at least four carbon atoms.

539. The process according to claim 331, wherein said Sig detectable moiety comprises an aromatic or cycloaliphatic group comprising at least five carbon atoms.

540. The process according to claim 331, wherein said aromatic or cycloaliphatic group is fluorescent or chemiluminescent.

541. The process according to claim 331, wherein said Sig detectable moiety comprises an aromatic or cycloaliphatic group comprising at least six carbon atoms.

542. The process according to claim 541, where said aromatic or cycloaliphatic group is fluorescent or chemiluminescent.

543. The process according to claim 331, wherein said Sig detectable moiety in said nucleotide (i) is covalently attached to said BASE at a position when BASE is a pyrimidine that is selected from the group consisting of the C2 position, the N3 position, the C6 position, and combinations thereof, or is covalently attached to BASE at a position when BASE is a purine that is selected from the group consisting of the N1 position, the C2 position, the N3 position, the C6 position, the N7 position, and combinations thereof.

544. The process according to claim 331, wherein said Sig detectable moiety in said nucleotide (i) is covalently attached to said BASE at a position selected from the group consisting of the N4 position when said pyrimidine comprises cytosine, the N2 position when

said purine comprises adenine or deazaadenine, the N6 position when said purine comprises guanine or deazaguanine, and combinations thereof.

552. The process according to claim 337, wherein said Sig detectable moiety in said nucleotide (i) is covalently attached to said BASE at a position selected from the group consisting of the N4 position when said pyrimidine comprises cytosine, the N2 position when said purine comprises adenine or deazaadenine, the N6 position when said purine comprises guanine or deazaguanine, and combinations thereof.

553. The process according to claim 348, wherein said Sig detectable moiety comprises an aliphatic chemical moiety comprising at least three carbon atoms and at least one double bond.

554. The process according to claim 348, wherein said Sig detectable moiety comprises an aliphatic chemical moiety comprising at least four carbon atoms.

555. The process according to claim 348, wherein said Sig detectable moiety comprises an aromatic or cycloaliphatic group comprising at least five carbon atoms.

556. The process according to claim 555, wherein said aromatic or cycloaliphatic group is fluorescent or chemiluminescent.

557. The process according to claim 348, wherein said Sig detectable moiety comprises an aromatic or cycloaliphatic group comprising at least six carbon atoms.

558. The process according to claim 557, wherein said aromatic or cycloaliphatic group is fluorescent or chemiluminescent.

559. The process according to claim 348, wherein said Sig detectable moiety in said nucleotide (i) is covalently attached to said BASE at a position when BASE is a pyrimidine that is selected from the group consisting of the C2 position, the N3 position, the C6 position, and combinations thereof, or is covalently attached to BASE at a position when BASE is a purine that is selected from the group consisting of the N1 position, the C2 position, the N3 position, the C6 position, the N7 position, and combinations thereof.

560. The process according to claim 348, wherein said Sig detectable moiety in said nucleotide (i) is covalently attached to said BASE at a position selected from the group consisting of the N4 position when said pyrimidine comprises cytosine, the N2 position when said purine comprises adenine or deazaadenine, the N6 position when said purine comprises guanine or deazaguanine, and combinations thereof.

561. The process according to claim 522, wherein said Sig detectable moiety comprises an aliphatic chemical moiety comprising at least three carbon atoms and at least one double bond.

562. The process according to claim 522, wherein said Sig detectable moiety comprises an aliphatic chemical moiety comprising at least four carbon atoms.

563. The process according to claim 522, wherein said Sig detectable moiety comprises an aromatic or cycloaliphatic group comprising at least five carbon atoms.

564. The process according to claim 563, wherein said aromatic or cycloaliphatic group is fluorescent to chemiluminescent.

565. The process according to claim 522, wherein said Sig detectable moiety comprises an aromatic of cycloaliphatic group comprising at least six carbon atoms.

566. The process according to claim 565, wherein said aromatic or cycloaliphatic group is fluorescent or chemiluminescent.

567. The process according to claim 522, wherein said Sig detectable moiety in said nucleotide (i) is covalently attached to said BASE at a position when BASE is a pyrimidine that is selected from the group consisting of the C2 position, the N3 position, the C6 position, and combinations thereof, or is covalently attached to BASE at a position when BASE is a purine that is selected from the group consisting of the N1 position, the C2 position, the N3 position, the C6 position, the N7 position, and combinations thereof.

568. The process according to claim 522, wherein, said Sig detectable moiety in said nucleotide (i) is covalently attached to said BASE at a position selected from the group

consisting of the N4 position when said pyrimidine comprises cytosine, the N2 position when said purine comprises adenine or deazaadenine, the N6 position when said purine comprises guanine or deazaguanine, and combinations thereof.